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TITLE: Engineering of Specific Tissue Inhibitors to Block ADAM Type Metalloprotease-Mediated Mammary Neoplasia

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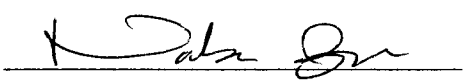

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13. ABSTRACT ***(Maximum 200 Words)***

Communication between different signaling pathways enables cells to coordinate the responses to diverse environmental signals. Activation of the transmembrane growth factor precursor plays a critical role in this communication and often involves in metalloproteases-mediated proteolysis. Stimulation of G protein-coupled receptors (GPCR) transactivates the epidermal growth factor receptors (EGFR), which occurs via a metalloprotease-dependent cleavage of heparin-binding epidermal growth factor (HB-EGF). However, the metalloprotease mediating the transactivation remains elusive. We show that the integral membrane metalloprotease Kuzbanian (KUZ, ADAM10), which controls Notch signaling by cleaving Notch and its ligand Delta in *Drosophila*, stimulates GPCR transactivation of EGFR. Upon stimulation of the bombesin receptors, KUZ increases the docking and activation of adaptors SHC and Gab1 on the EGFR, and activation of Ras and Erk. In contrast, transfection of a protease-domain deleted KUZ (K•MP) or blocking endogenous KUZ by morpholino antisense oligonucleotides suppresses the transactivation. The effect of KUZ on shedding of HB-EGF and consequent transactivation of the EGFR depends on its metalloprotease activity. GPCR activation enhances the association of KUZ and its substrate HB-EGF with tetraspanin CD9. Thus KUZ regulates the relay between GPCR and EGFR signaling pathways.

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Table of Contents

Introduction

Cells sense diverse extracellular signals with different types of receptors on cell surface, and transduce these signals into cells through distinct signal pathways. But there is also significant crosstalk between the signaling pathways. In many cases, crosstalk occurs at the level of sharing components in the pathway, such as MAP kinases and other intracellular kinases. Crosstalk also occurs directly between membrane receptors; for example, stimulation of G protein-coupled receptors (GPCR) leads to activation of epidermal growth factor receptors (EGFR) (Carpenter, 1999; Daub et al., 1996; Pierce et al., 2001a). This transactivation of EGFR by GPCR occurs via a release of heparin-binding epidermal growth factor (HB-EGF) which is blocked by metalloprotease inhibitor (Prenzel et al., 1999).

Metalloproteases regulate cell behavior by modifying both the macro- and microenvironment of cells during the normal growth and development (Werb, 1997; Werb and Yan, 1998). Misregulation of metalloprotease activities contributes to many pathological processes, including tumorigenesis (Sternlicht et al., 1999). The integral membrane metalloproteases with a disintegrin domain (ADAMs) cleave various membrane-bound proteins, including ligands, receptors and ligand-receptor complexes (Black and White, 1998; Blobel, 2000). Among the well studied of ADAMs are TNF-α-converting enzyme (TACE, ADAM17), which not only cleaves TNF-α but also converts pro-TGFα precursors to active TGFα (Peschon et al., 1998), and Kuzbanian (ADAM10), which is a key regulator of Notch signaling pathways in *Drosophila* because it cleaves Notch receptor and its ligand Delta (Pan and Rubin, 1997; Qi et al., 1999). Thus, proteolysis by ADAMs can change the active state of surface molecular complexes, affecting the signaling pathways inside cells.

Metalloprotease-mediated release of EGFR ligands not only contributes to normal development process, as revealed with the metalloprotease-deficient mice, but also plays important roles in abnormal growth of tumor cells. Inhibition of metalloprotease-mediated the EGFR ligand shedding reduces the proliferation and migration of breast cancer cells (Dong et al., 1999). Moreover, so-called autocrine growth of tumor cells is often EGFR-dependent, and GPCR ligands such as bombesin also act as growth factors. In PC3 prostate cancer cells, the metalloprotease inhibitor BB94 inhibits bombesin and TPA –induced EGFR transactivation (Prenzel et al., 1999). Thus metalloproteases are an integral part of this EGFR-dependent autocrine growth pathway. However, the understanding of pathway from GPCR to EGFR activation has been limited by the lack of knowledge about the metalloprotease involved. Several

metalloproteases are capable of cleaving EGFR ligands, but there is no evidence that they support the EGFR transactivation by GPCR. For example, ADAM9 cleaves the HB-EGF when PKCd is activated, but neither the wildtype, nor the dominant-negative ADAM9, affects the EGFR transactivation (Izumi et al., 1998; Prenzel et al., 1999). Here we report that the metalloprotease KUZ (ADAM10), described initially as the regulator of Notch signaling, supports the GPCR – induced transactivation of EGFR signaling pathway.

Body

To identify the metalloprotease that mediates the transactivation of EGFR, we transfected COS7 cells with mouse KUZ and other metalloprotease-disintegrins (ADAMs), and examined the EGFR activation after activating the transfected cells with GPCR ligands. Stimulation of COS7 cells with the GPCR ligands lysophosphatidic acid (LPA) and bombesin significantly increased EGFR activation, confirming that, indeed, there were strong interactions between the GPCR and EGFR signaling pathways (Fig. 1, A and B). Transfection of KUZ further augmented GPCR-induced EGFR phosphorylation. However, transfection of evolutionarily related ADAMs 9, 15, and TACE (ADAM17) did not elevate the EGFR activation by GPCR in COS7 cells (data not shown). These results suggest that KUZ enhances EGFR transactivation by GPCR.

In contrast to transfection of wild type KUZ, transfection of COS7 cells with a KUZ mutant, which lacks the metalloprotease domain (KΔMP) and acts as a dominant-negative mutation in *Drosophila*, reduced the GPCR-induced EGFR phosphorylation. In fact, after bombesin treatment, the level of EGFR phosphorylation in the KΔMP-transfected cells was lower than that in cells transfected with a vector plasmid, indicating that KΔMP blocks the endogenous metalloprotease-mediated EGFR transactivation in COS7 cells. In cells transfected with KUZ, the phosphorylation of EGFR in the presence of GPCR ligand was effectively inhibited by CRM197, an inactivated diphtheria toxin that specifically binds to HB-EGF, and the EGFR kinase inhibitor AG1478, confirming that effect of KUZ on increased EGFR phosphorylation is due to activation of EGFR ligand HB-EGF by GPCR signaling (Fig. 1 C). Thus KUZ appears to function as an intermediate between the activated GPCR and EGFR phosphorylation.

To access the effects of KUZ on signaling pathways downstream of transactivated EGFR, we investigated the docking and phosphorylation of proteins known to associate with phosphorylated EGFR. Transfection of KUZ increased, and transfection of KΔMP decreased the bombesin-dependent phosphorylation of all three isoforms of the adapter protein SHC (Fig. 2 A) and another adapter Gab1 (Fig. 2 B). KUZ, but not KDMP, increased amount of phosphorylated EGFR co-precipitated with

SHC upon bombesin treatment (data not shown), indicating that KUZ facilitated the direct recruitment of SHC by activated EGFR. Thus KUZ increases activation of signaling components docked on activated EGFR upon stimulation of GPCR.

GPCRs such as bombesin receptor activate growth signaling through both Ras-dependent and independent pathways. To test whether KUZ-mediated transactivation of EGFR is an integral part of the mechanism that GPCR activates Ras pathway, we next examined the effect of KUZ on the transactivation of Ras, which links the activated EGFR to MAP kinase activation. Precipitation of activated Ras from the COS7 cell lysates with the fusion protein containing the Ras-binding domain of Raf1 showed that bombesin treatment significantly increased Ras activation (Fig. 2 C). The expression of KUZ, but not KΔMP further elevated the increase of activated Ras. The results demonstrate that the metalloprotease KUZ contributes to the GPCR activation of EGFR, which leads to activation of the Ras-dependent signaling pathway.

Antagonists of bombesin inhibit tumor growth in nude mice seeded with PC3 cells and their effects are EGFR-dependent, suggesting EGFR is involved in bombesin-induced tumor growth (Heasley, 2001; Plonowski et al., 2000). Interestingly, the expression of ADAM10, the human homologue of KUZ significantly increases with androgen treatment in PC3 cells, while TACE expression is inhibited (McCulloch et al., 2000). To assess whether endogenous KUZ was involved in GPCR transactivation of EGFR signaling pathway, we then investigated the effect of KUZ on activation of MAP kinase (Erk1/2) in PC3 prostate cancer cells. Inhibition of EGFR kinase with AG1478 and neutralization of HB-EGF with CRM197 both reduced Erk1/2 phosphorylation by bombesin, suggesting that transactivation of EGFR is required, at least in part, for bombesin to activate Erk signaling in PC3 cells (Fig. 2 D).

To determine whether PC3 cells require KUZ for this transactivation, we used two methods to inhibit endogenous KUZ and tested the response of the cells to bombesin. First, transfecting KΔMP suppressed transactivation of Erk1/2 by bombesin (Fig. 2 E). Second, introduction of the specific antisense morpholino oligonucleotide against ADAM10 inhibited the bombesin-mediated transactivation of Erk1/2, while a control oligonucleotide with same nucleotide sequence but in a reverse orientation did not (Fig. 2 F), nor did the anti-sense oligonucleotides against ADAMs 9, 15, and 17 (data not shown). These results show that blocking KUZ inhibits transactivation, implicating the endogenous KUZ is a critical player in GPCR transactivation of EGFR signaling.

The effect of KUZ on the EGFR transactivation required its metalloprotease activity. KUZ was involved in shedding of HB-EGF from COS7 cells. Stimulation of cells with bombesin increased the amount of shed HB-EGF in the medium. KUZ enhanced the ability of bombesin to release HB-EGF into medium (Fig. 3 A). In contrast, KΔMP

blocked the release of HB-EGF. These data show that KUZ is required to shed HB-EGF. Inhibition of metalloprotease using a broad-spectrum inhibitor TAPI also inhibited bombesin-induced EGFR and SHC phosphorylation (Fig. 3 B). A single E385A mutation causes the loss of KUZ metalloprotease activity. When this mutant K(E-A) was introduced in COS7 cells, EGFR and SHC activation did not increase upon bombesin treatment (Fig. 3 C). Therefore, metalloprotease activity of KUZ is responsible for EGFR transactivation.

However, the ability to cleave HB-EGF alone is not sufficient to cause the transactivation; an additional signaling step is needed. Indeed, ADAM9 cleaves the HB-EGF under the regulation of PKC, yet neither the wild type nor the dominant-negative ADAM9 affects the GPCR-induced EGFR phosphorylation (Izumi et al., 1998; Prenzel et al., 1999). Stimulation of GPCR unleashes an array of signaling mediators that may activate KUZ to support the EGFR transactivation. Interestingly, only Gi and Gq-coupled GPCRs are associated with transactivation of EGFR and these GPCRs activate p44/42 Erk. Whether Erk directly regulates the cleavage of proTGFa (Fan and Derynck, 1999) and c-Met (Nath et al., 2000) in response to extracellular signals is unresolved, because dominant negative MEK1 does not block the transactivation resulted from HB-EGF shedding (Pierce et al., 2001b). However, inhibition of Src kinase has a profound effect on transactivation (Pierce et al., 2001b). It is conceivable that kinases, such as Src activated by GPCR, can phosphorylate cytoplasmic domain of ADAMs. The mechanism by which this phosphorylation activates metalloprotease remains elusive because the cytoplasmic domain of TACE is dispensable in PMA induced shedding (Reddy et al., 2000).

For shedding to occur *in cis* would require binding of KUZ to its substrate proHB-EGF. We therefore hypothesized that GPCR affects KUZ activity by regulating the formation of such a complex. Indeed, we found that the antibody to CD9, a tetraspan-transmembrane protein, precipitated not only HB-EGF, but also KUZ from cell lysates, suggesting they co-exist in the same molecular complex on the cell membrane (Fig. 4). Precipitation of CD9 co-precipitated with two forms of HB-EGF, the long form of about 24 kD and a short form of 18 kD. While CD9 binding of the long form appeared to be constitutive, the complex of short form with CD9 increased in the presence of transfected KUZ and with bombesin treatment. Therefore GPCR regulates KUZ-dependent activation of HB-EGF by promoting the binding of KUZ to the molecular complex centered around CD9. Interestingly, the potency of HB-EGF in stimulating cell growth correlates with its binding to CD9. CD9 also associates with ADAM2 and regulates interaction of ADAM2 with $\alpha 6\beta 1$ integrin (Chen et al., 1999; Shi et al., 2000).

We have shown that metalloprotease KUZ defines a control point in the relay between the GPCR and the EGFR signaling pathways. The identification of KUZ as the mediator of transactivation reveals an evolutionarily conserved role of KUZ in coordinating cell behavior. EGFR transactivation occurs not only in the same cell where both KUZ and EGFR reside, but also affects the neighboring cells that sense released HB-EGF. By relaying the extracellular signal from GPCR to EGFR, KUZ not only propagates signal laterally on the cell membrane, but also coordinates the response of surrounding cells with cleaved HB-EGF. Interestingly, two other well defined KUZ functions also involve precise control of signals between adjacent cells: in lateral inhibition where KUZ mediates cleavage of Notch and its ligand Delta (Pan and Rubin, 1997; Qi et al., 1999), and in interaction and cleavage of ephrin-A2 upon binding to its receptor Eph in the contacting cell (Hattori et al., 2000). In all cases, KUZ functions at the point of cell-cell contact. Elucidation of the mechanism of GPCR activation of KUZ increases our understanding of how metalloprotease-mediated shedding regulate signaling pathways.

Figures:

Figure 1 **KUZ mediates GPCR transactivation of EGFR.** (A) KUZ, but not KΔMP, stimulates the LPA induced EGFR phosphorylation. (B) KUZ, but not KΔMP, stimulates bombesin induces EGFR phosphorylation. EGFR was immunoprecipitated from GPCR stimulated or control cells transfected with KUZ, KDMP and vector DNA. Activation of EGFR was detected in immunoblots as phosphorylation of EGFR with anti-phosphotyrosine antibody 4G10 and amount of EGFR was detected with goat anti-EGFR antibody against EGFR. (C) bombesin-induced EGFR phosophorylation depends on HB-EGF and EGFR kinase. COS7 cells transfected with bombesin receptor and KUZ were pretreated for 20 min with CRM197 (lanes 3 and 4) or specific EGFR kinase inhibitor AG1487 (lanes 5 and 6) before treatment with Bombesin. EGFR was precipitated from cell lysates. EGFR activation was detected in immunoblots with anti-phosphotyrosine antibody 4G10 and amount of EGFR was detected with goat anti-EGFR antibody in the same blot.

Figure 2 **KUZ stimulates, and blocking endogenous KUZ inhibits the transactivation of signaling pathway downstream of EGFR**. (A) Activation of all three forms of SHC by GPCR is stimulated by transfecting KUZ, but not protease-deleted KΔMP. (B) GPCR-induced Gab1 phosphorylation are stimulated by KUZ, but not KΔMP. SHC or Gab1 in COS7 cells was immunoprecipitated with the polyclonal

antibody to SHC or Gab1. Activation of SHC or Gab1 was detected in immunoblots as phosphorylation of SHC or Gab1 with anti-phosphotyrosine antibody 4G10. (C) KUZ elevates GPCR-induced Ras activation. The activated Ras in cell lysates was selectively absorbed onto beads coated with RBD domain of Raf1. The amounts of activated Ras in the cells were detected in the immunoblot with anti-Ras antibody. The upper band non-specifically reactive to the anti-Ras antibody is shown as the loading control. (D) Bombesin induces Erk1/2 activation that is partly due to transactivation of EGFR and depends on HB-EGF release. PC3 cells were treated with CRM197 or AG1487 and then stimulated with bombesin. Erk1/2 activation in treated or control PC3 cells were detected with anti-phospho-Erk1/2 in immunoblots of cell lysates. (E) Transfecting KΔMP suppresses Erk1/2 activation in PC3 cells. Bombesin treated or untreated cells PC3 cells transfected with vector or KΔMP were lysed and active Erk1/2 were detected in immunoblots with anti-phospho-Erk1/2 antibody. (F) Bombesin-induced Erk1/2 activation can be inhibited by an anti-sense morpholino oligo against ADAM10. Morpholino anti-ADAM10 oligos (Anti-KUZ) and control oligos with the same base composition but in reverse order (Reverse-KUZ) were introduced into PC3 cells. Erk1/2 phosphorylation was detected in lysates of PC3 cells treated with bombesin. The endogenous ADAM10 in antisense oligo-treated cells was detected with polyclonal antibody to ADAM10 (KUZ).

Figure 3 **Metalloprotease activity of KUZ is responsible for the GPCR transactivation EGFR signaling pathway.** (A) Transfection of wild-type KUZ, but not KΔMP, stimulates the release of soluble HB-EGF into the medium. The HB-EGF in the medium of cells transfected with HA-tagged HB-EGF and KUZ or KΔMP was analysed by collecting the heparin-binding proteins with heparin-agarose following stimulation with bombesin. The HA-tagged HB-EGF in collected protein pool was detected with anti-HA antibody 12CA5. (B) Metalloprotease inhibitor TAPI blocks bombesin-induced transactivation of EGFR and SHC. Cells pre-incubated either with TAPI or solvent DMSO before stimulation with bombesin. EGFR or SHC was immunoprecipitated and immunoblotted with anti-phosphotyrosine antibody 4G10 to reveal the activated EGFR and SHC. The total EGFR and SHC were equivalent in each lane (not shown). (C) Catalytically-inactive KUZ (K(E-A)) does not support the EGFR and SHC transactivation. EGFR or SHC were precipitated from cleared cell lysates and immunoblotted with anti-phosphotyrosine antibody 4G10 to reveal the activated EGFR and SHC. The total EGFR and SHC were equivalent in each lane (not shown).

Figure 4 **GPCR activation regulates the formation of a complex of CD9 with KUZ and HB-EGF**. COS7 cells were transfected with Flag-tagged KUZ and HA-tagged HB-EGF. CD9 was precipitated from CHAPS lysates of control or bombesin-treated cells with a polyclonal anti-CD9 antibody. Proteins from immunoprecipitation were blotted with monoclonal antibodies against Flag epitope, HA epitope, and CD9, respectively

Figure 1

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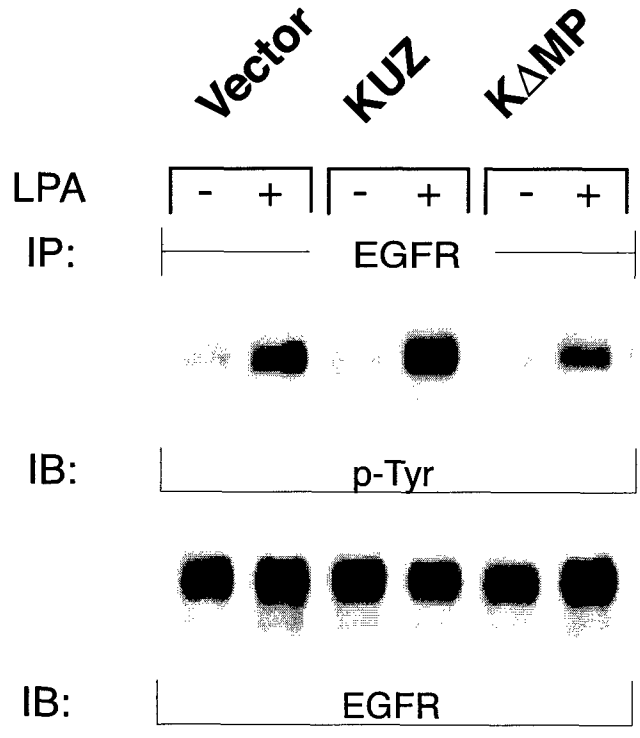

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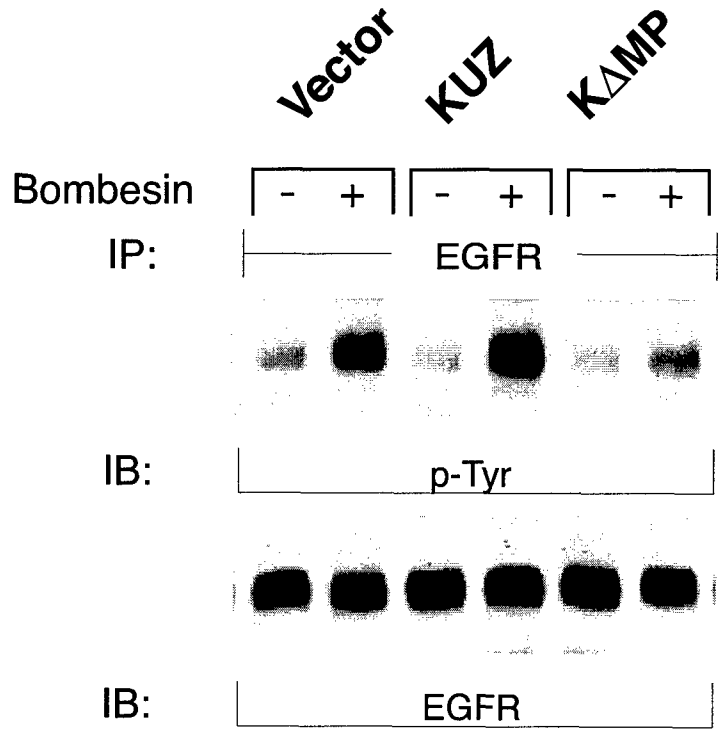

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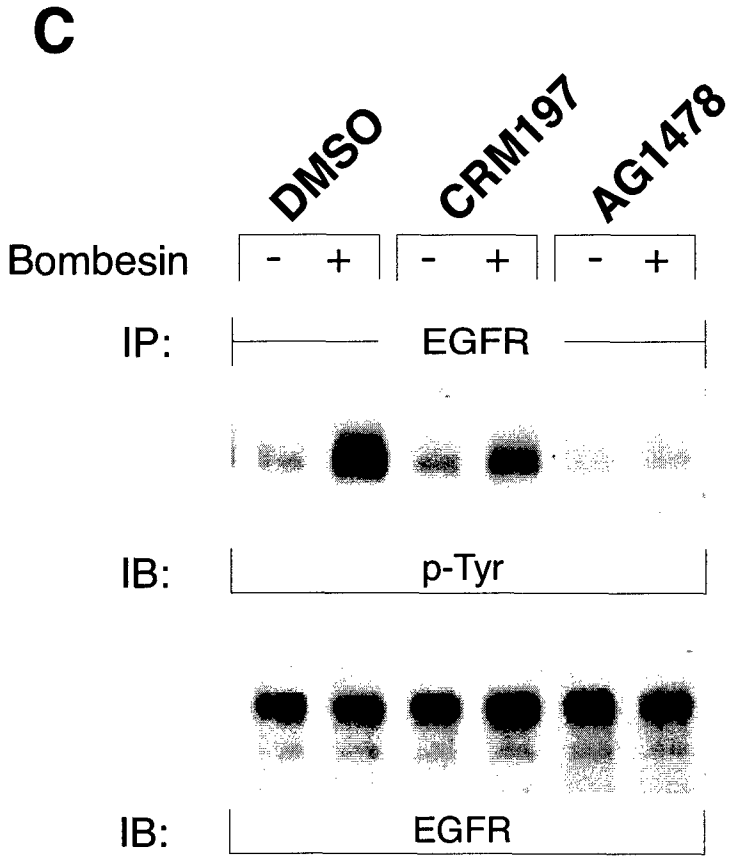

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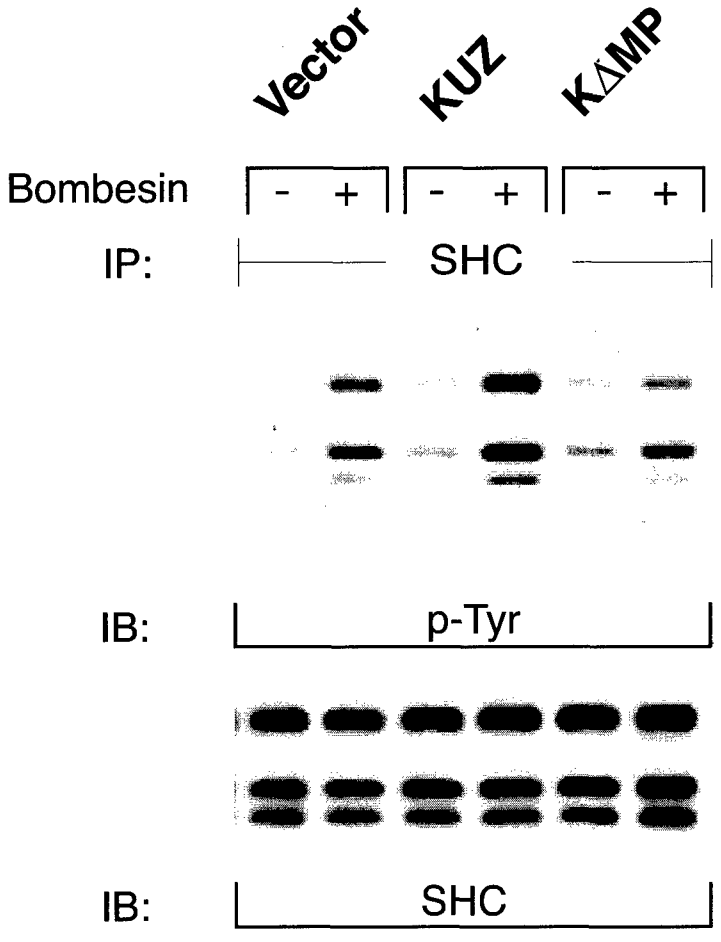

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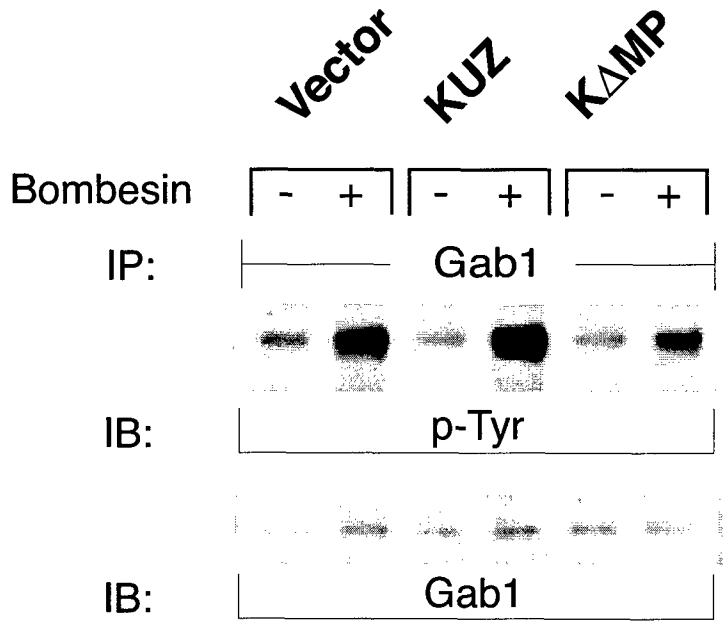

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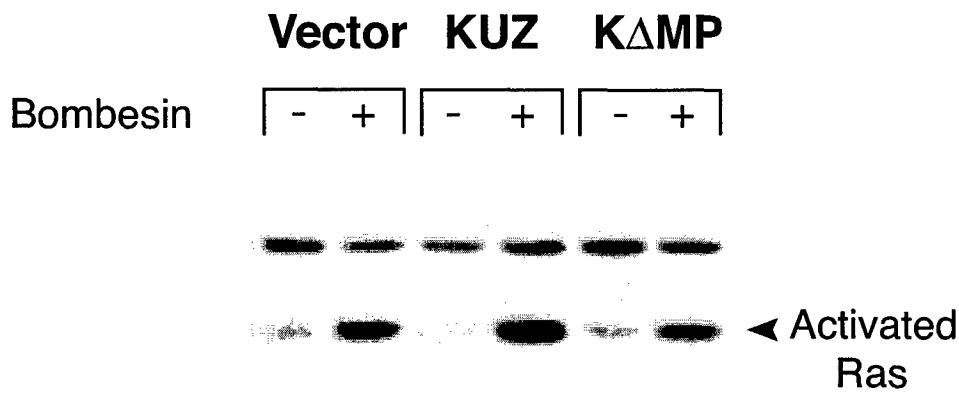

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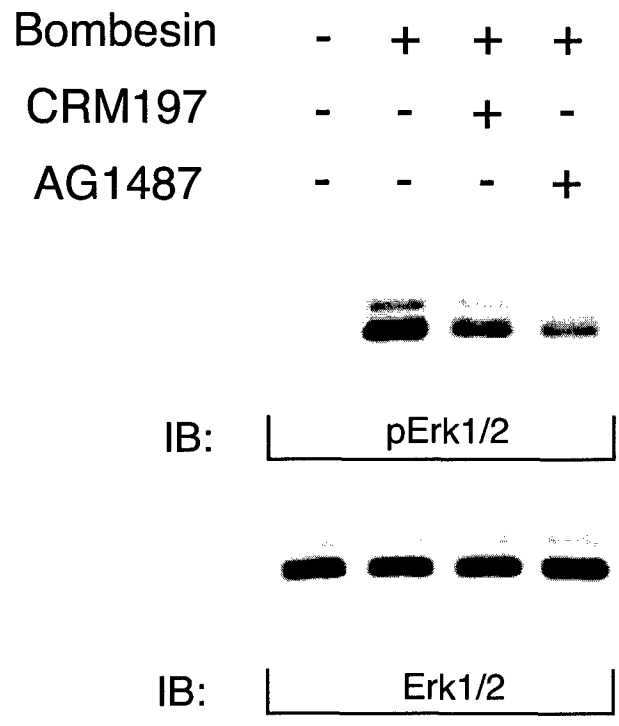

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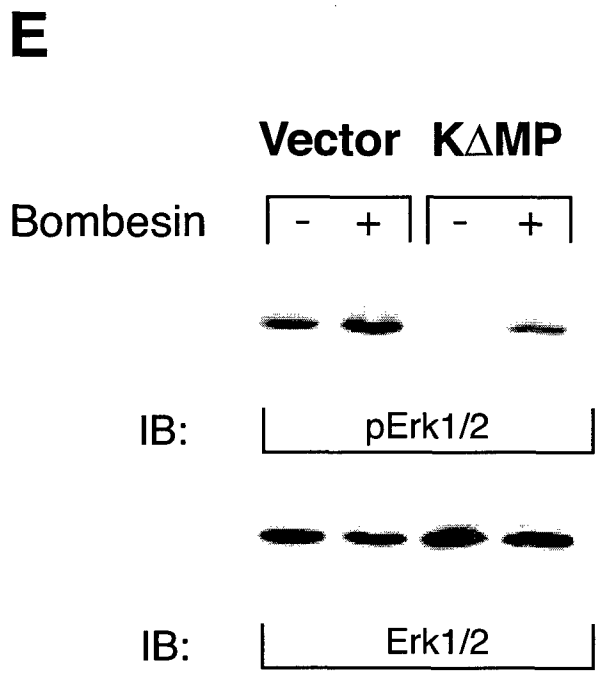

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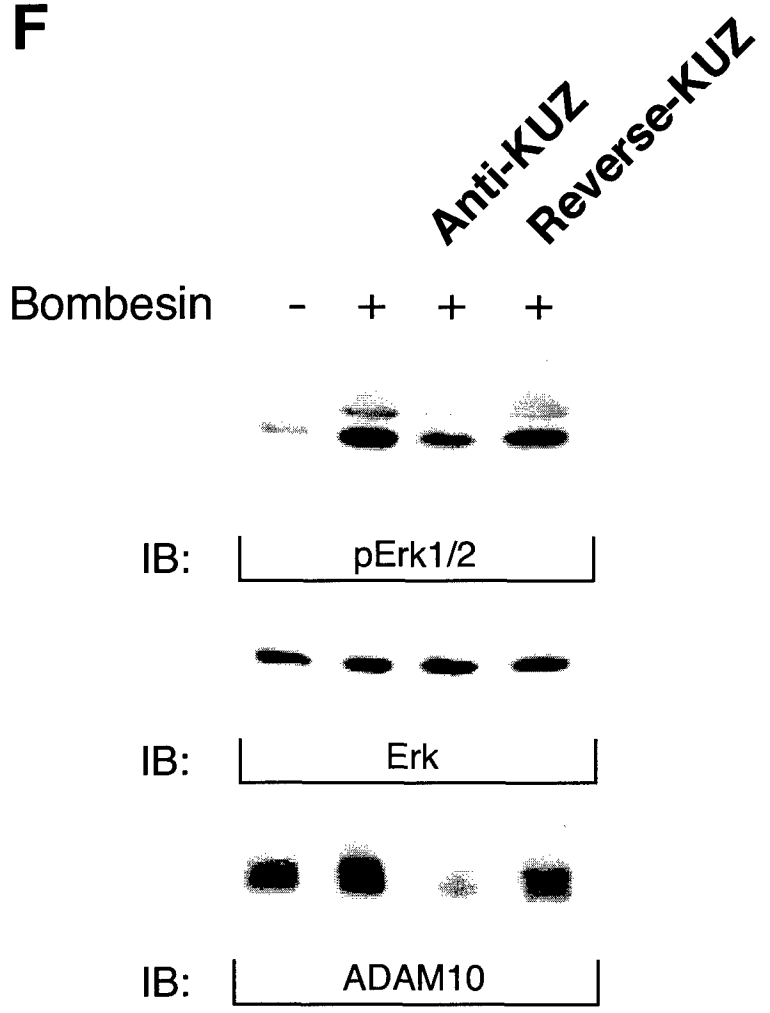

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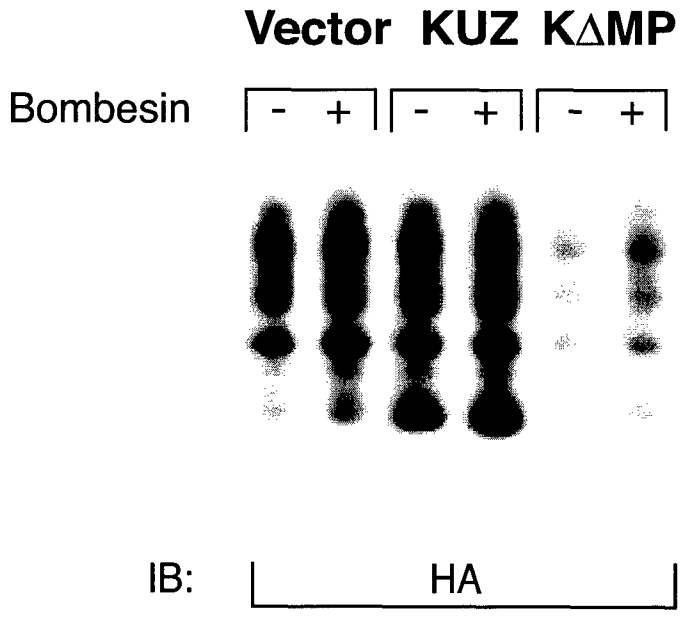

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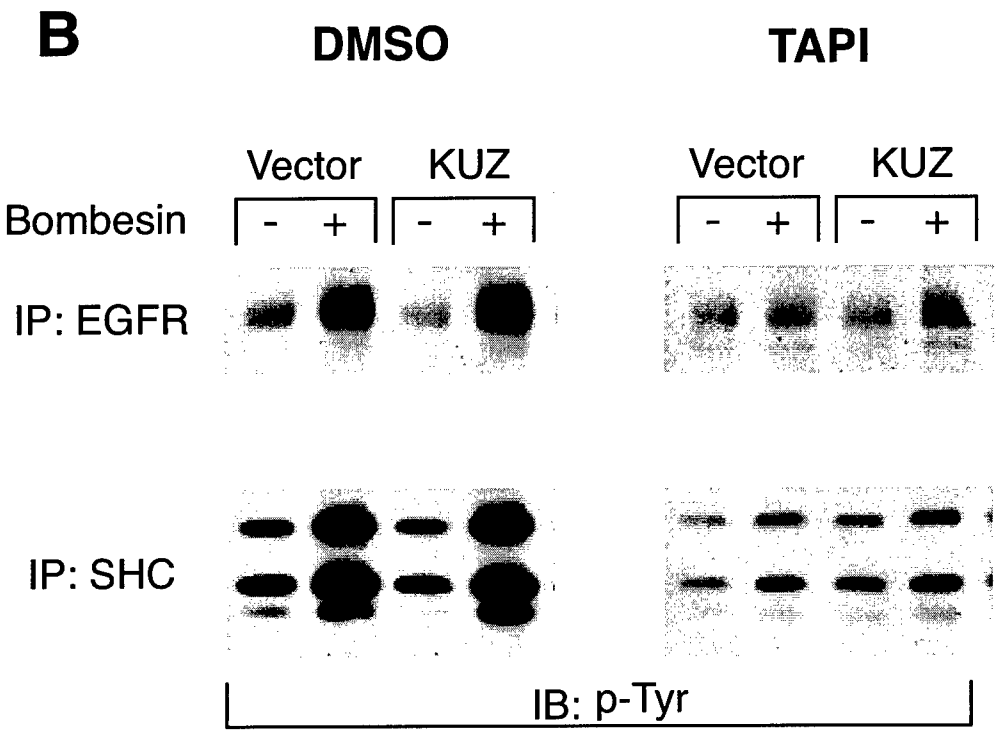
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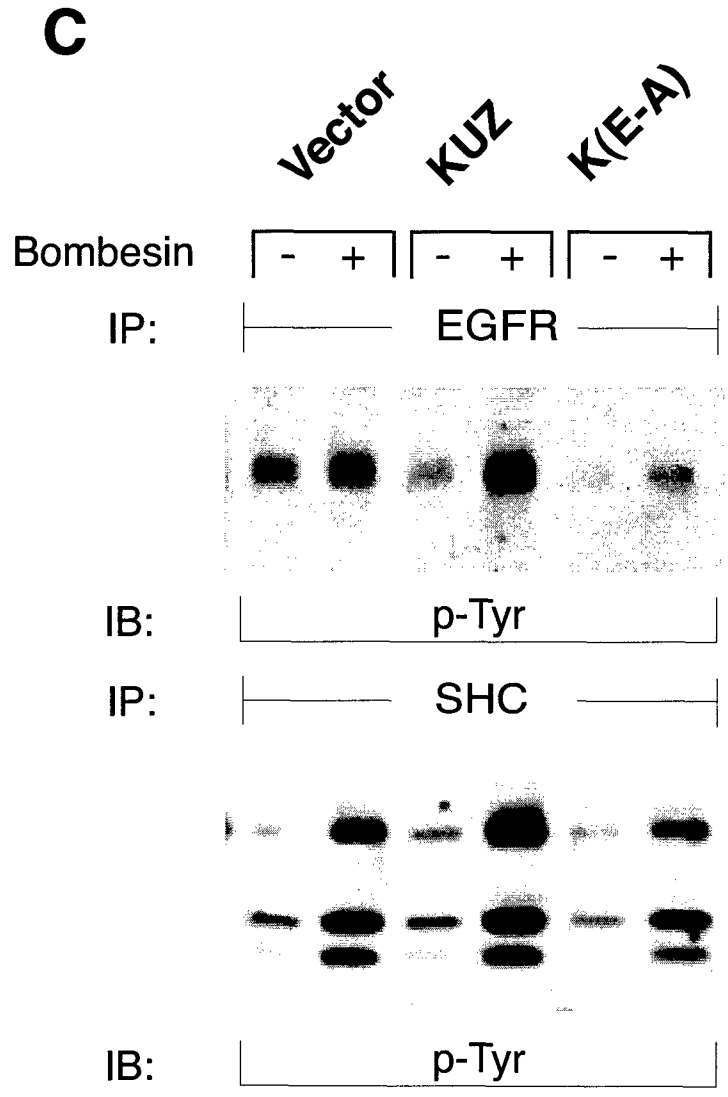
Figure 3



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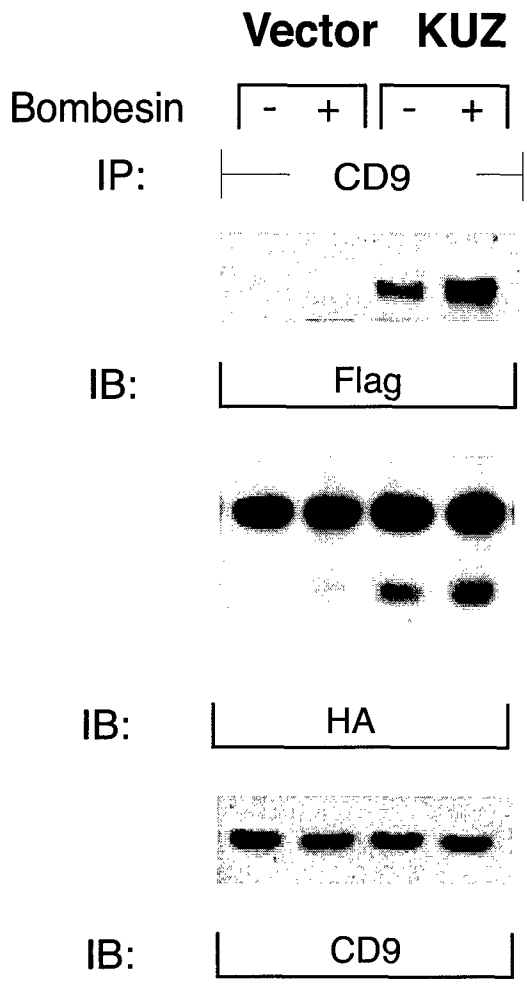

Key Research Accomplishments

Demonstrate that the integral membrane metalloprotease KUZBANIAN (ADAM10) mediates the signal relay between G-protein coupled receptors and EGF receptors, which may play a key role in sustaining the outocrine growth of late stage breast cancer cells.

Reportable Outcomes

Publication: Werb Z., and Yan Y. 1998. A cellular striptease act. *Science*. 282:1279-80.

Two selected oral presentation:

42th Annual Meeting of American Society of Cell Biology. 2001, San Francisco.

Gordon Research Conference in Matrix Metalloproteinases, 2001, Il Ciocco, Italy,

Also invited to give a speech at the Frontier of Metalloproteases and TIMPs Research in Tokyo, Japan, Oct. 2001 (the event was cancelled due to the War on Terrorism.)

Three transgenic mice lines:

TRE-DNK (dominant-negative KUZ),

TRE-TIMP3cs (TIMP3 inactive mutant), and

TRE-INT3 (constitutively active Notch4 mutant).

Conclusions

The proposed research seeks to validate the concept of blocking integral membrane proteases Kuzbanian to suppress neoplastic growth of mammary cells. We have shown that metalloprotease KUZ defines a control point in the relay between the GPCR and the EGFR signaling pathways. EGFR-mediated autocrine signaling palys a key role in neoplastic growth of breast cancer cells, where many GPCR ligands act as autocrine growth factors. By relaying the extracellular signal from GPCR to EGFR, KUZ not only propagates signal laterally on the cell membrane, but also coordinates the response of surrounding cells with cleaved HB-EGF. The identification of KUZ as the mediator of EGFR transactivation not only reveals an evolutionarily conserved role of KUZ in coordinating cell behavior but also unveils the key step in the autocrine growth of tumor cells through metalloprotease-mediated cleavage of EGFR ligand HB-EGF.

The results of complete research validates the integral membrane metalloprotease ADAM10 (KUZ) as a target for control the growth of unregulated breast cancer cells, and directly suggest that a specific inhibitor of ADAM10 (KUZ) may be valuable in suppressing late stage breast cancer growth.

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Appendices

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SCIENCE

A Cellular Striptease Act

Zena Werb and Yibing Yan

A Cellular Striptease Act

Zena Werb and Yibing Yan

The cell surface is a dynamic place. During its life history the cell alters the repertoire of proteins displayed on its surface many times. Membrane-anchored adhesion molecules, receptors, ligands, and enzymes are removed and replaced as the cell proceeds through development and as its activation state changes. How is this wholesale refurnishing of the cell membrane orchestrated? One key mechanism is proteolytic processing of the ectodomain (extracellular domain) of such membrane proteins. Cleavage or shedding of the ectodomains of plasma membrane proteins—widely observed in cells in culture—is blocked by inhibitors of metalloproteinases (*1, 2*). This result suggests that transmembrane and soluble metalloproteinases, such as matrix metalloproteinases (MMPs) and their relatives, are rate-limiting for cleavage and shedding. Other evidence also implicates serine proteinases in these processing events (*3, 4*).

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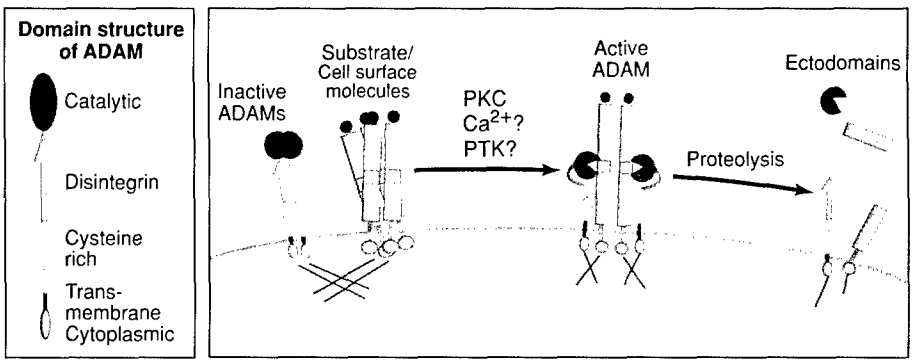
The first such "sheddase" characterized was the tumor necrosis factor–α (TNF-α) converting enzyme (TACE) (*5*). The study by Peschon and colleagues (*6*) on page 1281 of this issue now points to TACE's essential role in the shedding of ectodomains during mouse development. The surprise comes from the observation that mice lacking TACE do not show a phenotype indicative of a lack of TNF-α availability. Rather, they show the same phenotype as mice engineered to be without the epidermal growth factor (EGF) receptor—because TACE-mediated proteolysis makes available ligands for the EGF receptor, particularly transforming growth factor–α (TGF-α).

TACE turns out to be a membrane-anchored proteinase that is a member of the ADAM (a disintegrin and metalloproteinase) domain family of proteins that combines features of both cell surface adhesion molecules and proteinases (*8*). ADAMs all have a common domain organization, which endows these proteins with several potential functions—proteolysis, adhesion, signaling, and fusion (see figure below). The proteolytically competent ADAMs, such as TACE (ADAM17), are zinc-dependent metalloproteinases, closely related to the MMP family with which they share small molecule inhibitors and even one tissue inhibitor, TIMP-3 (*9, 10*). Several newly discovered MMPs appear to be hybrids of both MMP and ADAM domains (*11*), indicating that these two types of enzymes are part of one, larger family.

The ADAM proteinases are themselves targets of proteolytic events that ultimately strip off the catalytic domains (*5, 8*). This action could be a mechanism of functionally blunting the effects of the proteinases (see the figure on the next page). These soluble ADAMs may have proteolytic activity, as is the case for snake venom enzymes (*8*), but soluble TACE is much less active than membrane-bound enzyme (*5, 6*). The residual adhesive domains of ADAMs left after cleavage may have regulatory or adhesive functions. In support of this idea, a catalytic domain-deleted mutant of *Kuz* (ADAM10/SUP17), first identified as being required for cleavage of Notch during neural development in *Drosophila*, exerts a dominant negative effect (*8, 12*). During sperm maturation fertilin, a heterodimeric ADAM essential for sperm-egg interaction (*13*), also loses its catalytic domains by proteolytic processing. The remaining adhesive disintegrin domain is then competent to bind integrins.

How does TACE act? TACE is widely expressed in the animal. Mutation of the catalytic domain of TACE (*6*) reveals several distinct functions for this ADAM in development. Ligands for the EGF receptor, which is essential for epithelial development (*7*), are usually made and used locally (*14*). Although the growth factor precursors may have some biological activity (*15*), the new results imply that the membrane-anchored forms are essentially inactive precursors (*6*). TACE also cleaves ectodomains of other receptors and ligands, such as TNF-α, the p75 TNF receptor, and L-se-



Activation of sheddases. The ADAM proteases (as dimers) and substrates are anchored apart in the plane of the membrane. Upon activation (via protein kinases and other pathways) they are brought together and proteolysis takes place, leading to free ectodomains.

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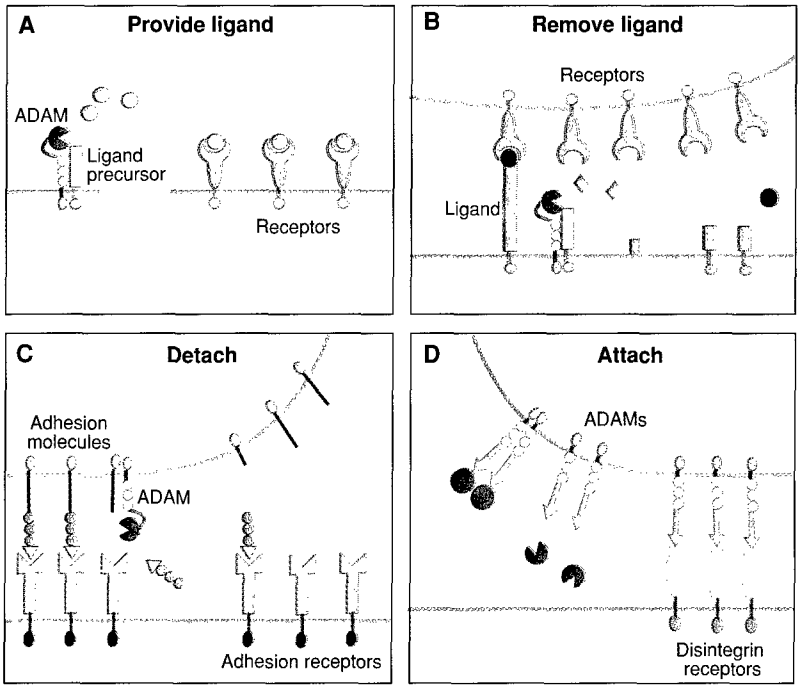
lectin, and thus participates in inflammatory and pathological reactions (*6*).

Processing membrane proteins by the ADAMs and other sheddases requires both the membrane-anchored enzyme and its substrate to be present in cis on the same cell (*6, 8, 12*). This presents several interesting problems. How are the active cell surface proteinases kept separate from their cell surface substrates until shedding is triggered? How do you exert selectivity for only certain ectodomain targets, out of many transmembrane proteins displayed on the cell surface? And how are the proteinase and substrate brought together in a coordinated manner so that all the cell surface substrate molecules can be removed within seconds, as occurs for the adhesion molecules L-selectin and syndecans (*4, 16*)?



Versatile shedding. Sheddases can supply or down-regulate ligands for receptors. Cleavage of adhesion molecules on cell surface or exposure of the disintegrin domain of ADAM regulate cell-cell and cell–extracellular matrix interactions.

Despite nonconserved cleavage sites that may be adjacent to the membrane or further out on the molecule, there are clues that a common strategy may operate in most cases. First, all ectodomain shedding is inhibited in a single mutant cell line (*1*). Second, the proteolysis is regulated in different cell types by activation of protein kinase C (PKC), calcium/calmodulin kinases, or receptor tyrosine kinases (*1, 17*). A model that accounts for these observations requires the processing proteinases and their transmembrane substrates both to be anchored in distinct domains of the plasma membrane, probably through cytoskeletal interactions (see figure on previous page). Upon cell activation, the attachments change and the proteinases and substrates become coclustered and can interact. Alternatively, the signaling cascade could modify the cytoplasmic domains of the proteinases or substrate, producing a conformational change that either activates the enzyme or makes the cleavage site available.

Although activation of the shedding reaction appears to control the rapid and complete removal of cell surface molecules such as L-selectin (an adhesion molecule involved in leukocyte rolling and extravasation into inflammatory sites) for most processing reactions there appears to be a constitutive level of ectodomain shedding. Processing is necessary to make available paracrine growth and survival factors such as TGF-α, EGF, HB-EGF, the kit ligand, and amphiregulin (*18*). This makes sense to allow for the consistent supply of growth factors (see figure above).

Endogenous inhibitors allow even finer control of the action of the shedding enzymes. Recently TACE was shown to be inhibited by TIMP-3, but not by the three other TIMPs that also inhibit MMPs (*10*). If TACE liberates a survival factor, then the presence of TIMP-3 could lead to cell death. This may explain why TIMP-3, but not other TIMPs, induce apoptosis (*19*).

Proteolysis of the ectodomains of growth factor coreceptors such as syndecan provide a second mechanism for regulating growth factor availability. Shedding the ectodomain of syndecan converts it to a potent inhibitor of FGF-2 (*20*). Just as shedding can make growth factor ligands available and control proliferation and survival, cleavage can also control cell death. Membrane-bound Fas ligand induces apoptosis by binding to the Fas receptor. Proteolysis functionally down-regulates the ligand and short-circuits apoptosis in lymphoid cell (*21*).

Cell surface adhesive molecules can also be regulated by proteolysis. An emerging paradigm is that cleavage of adhesive molecules not only alters adhesion, but completely revamps cell signaling. In the case of Notch, cleavage by *Kuz* is required to make it functional as a receptor, promoting adhesion, signaling, and cell lineage choices (*12*). Shedding of L-selectin by TACE or related enzymes inhibits leukocyte rolling and blunts their extravasation to inflammatory sites (*16*). The shedding of the ectodomains of E-cadherin (*22*) and transmembrane protein tyrosine phosphatases such as LAR have profound effects on cell-cell adhesions and also on important signaling pathways (*17*). These changing adhesion receptors and ligands may also be part of the apparatus for pathfinding in the nervous system.

Cells use a limited number of strategies to remodel their microenvironments. It is clear that the shedding process is an ancient, conserved, and fundamental pathway present from worms to humans. Thus, proteolysis by cell surface shedding enzymes provides a mechanism by which the wardrobe of externally displayed molecules can be changed or discarded. Spatial restriction of the enzymes and their substrates allows for either instant action or sustained activity.

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